

## CHART PRESENTATION ON RECENT WORK ON INDICATORS

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During recent years the change of mental attitude of scientific workers towards indicators has been very pronounced. The advent of the hydrogen electrode, and the results obtained by its use in the hands of Friedenthal<sup>1</sup> and Salm,<sup>2</sup> have resulted in the addition of data founded on a quantitative basis to the empirical rules of ten years ago. In a recent paper, S. P. L. Sørensen<sup>3</sup> has published the results of an investigation of the hydrogen ion concentration of various mixtures of pure substances and the corresponding colour changes of an exhaustive list of indicators.

*'Reaction' and 'Hydrogen ion Concentration'*

It will be necessary in the ensuing description to affix, for the time being at any rate, explicit meanings to some terms frequently employed. By means of hydrogen electrodes it is possible to determine directly the hydrogen ion concentration of a solution (expressed, of course, in terms of the normal hydrogen solution, one gram per litre). The value for any solution is conveniently expressed as a power of ten (that is to say, the common logarithm), and indicated by the symbol  $p_{\text{H}}^+$ , the negative sign being understood. For instance, centinormal hydrochloric acid is 0.00916 N with respect to its hydrogen ions, and the hydrogen ion

1. H. Friedenthal, *Zeitschr. f. Electrochemie*, Vol. X, p. 113, 1904.
2. Ed. Salm, *ibid.*, Vol. X, p. 341, 1904; Ed. Salm and H. Friedenthal, *ibid.*, Vol. XIII, p. 125, 1907, &c.
3. S. P. L. Sørensen, *Comptes Rendus des Travaux du Laboratoire de Carlsberg, Copenhagen*, Vol. VIII, pp. 1-168, 1909; Vol. VIII, pp. 396-401, 1909.

concentration is expressed conveniently as  $10^{-2.04}$  the index  $-2.04$  being the logarithm of  $0.00916$ , or it is said that the value of  $p_{\text{H}}^+$  for centinormal acid is  $2.04$ .

Now experiments on the inversion of saccharose by acids and other similar phenomena, the precipitation of colloids, the optimum conditions for enzyme actions, and so on, show distinctly that those properties which are common to all acid solutions, and which we usually put down to their 'acidity,' are quantitatively dependent upon the concentration of the hydrogen ions, i.e., the value of  $p_{\text{H}}^+$ .

With reference to indicators, it may be stated that when an indicator assumes a certain tint intermediate between those of its strongly acid and alkaline solutions, it shows, within limits of accuracy which experiment can determine, that the solution in which it is dissolved has a certain definite hydrogen ion concentration. Further, when another solution is observed to have the same tint under identical conditions of temperature and dilution, this second solution is found to have the same hydrogen ion concentration as the first.

In brief, the *absolute* hydrogen ion concentration can be measured in many ways, but most conveniently and directly by the potential set up between the solution and hydrogen: the *identity* of the hydrogen ion concentrations of two solutions can be established by the careful use of indicators.

The process of 'titration' is of quite another kind. It is the determination of the proportions in which two liquids must be mixed in order that the mixture may give, with an indicator, a certain result previously agreed upon—that is to say, in order that the mixture may have a certain value of  $p_{\text{H}}^+$ .

It must be fully realised that the titration values (c.c. of N/10 alkali to be added per 10 c.c. of solution, let us say, in order to obtain a mixture having value  $p_{\text{H}}^+ = 5.1$ ) of a number of solutions having, say,  $p_{\text{H}}^+ = 1.3$ , may be very different indeed. A solution of glycocoll and hydrochloric acid having  $p_{\text{H}}^+ = 1.3$  will need far more caustic soda to bring the value of  $p_{\text{H}}^+$  to  $5.1$  than will a solution of pure hydrochloric acid having the same hydrogen ion concentration.

*The Chart compiled from curves and tables given in Sørensen's Paper*

Sørensen's results can be conveniently displayed by means of the accompanying diagram, in which the behaviour of indicators towards

mixtures of known hydrogen ion concentration is graphically represented, and which I have constructed from the various tables and curves given in the original paper.

The following solutions were prepared:—

- (1) Decinormal hydrochloric acid, designated in the drawing simply 'HCl.'
- (2) Decinormal caustic soda, 'NaOH.'
- (3) A decinormal solution of glycocoll containing in 1 litre 7.505 grams glycocoll and 5.85 grams sodium chloride, 'glycocoll.'
- (4) 1/15 molecular primary potassium phosphate containing in 1 litre 9.078 grams, 'KH<sub>2</sub>PO<sub>4</sub>.'
- (5) 1/15 molecular secondary sodium phosphate containing in 1 litre 11.876 grams, 'Na<sub>2</sub>HPO<sub>4</sub>.'
- (6) 1/10 molecular secondary sodium citrate containing in 1 litre 21.008 grams hydrated citric acid and 200 c.c. of normal caustic soda, 'Na<sub>2</sub>H Cit.'
- (7) A solution containing in 1 litre 12.404 grams boric acid and 100 c.c. of normal caustic soda, 'NaH<sub>2</sub>BO<sub>3</sub>.'

These are from pure chemical substances, and can be re-made at any time. The values of  $p_{\text{H}}^+$  were determined for these solutions and for binary mixtures of them, and the results are plotted in the middle portion of the diagram. Each curve represents one binary mixture prepared from two of the above solutions. The ordinate at any point in a curve represents the value of  $p_{\text{H}}^+$  and the abscissa the number of parts, in ten of the mixture, of the 'less acid' or 'more alkaline' constituent.

For instance, in the case of the curve for a mixture of 'glycocoll' solution and deci-normal 'hydrochloric acid' solution the ordinate at A ( $p_{\text{H}}^+ = 1.04$ ) represents the value of  $p_{\text{H}}^+$  for 'hydrochloric acid,' and that at B ( $p_{\text{H}}^+ = 6.11$ ) value for the 'glycocoll' solution (the less acid of the two constituents), and that at C ( $p_{\text{H}}^+ = 2.6$ ), the value for a mixture of 3 c.c. of the N/10 'hydrochloric acid' and 7 c.c. of the 'glycocoll' solution.

Conversely from the curves can at once be read what solutions and what proportions of them must be taken to obtain a mixture of any desired value of  $p_{\text{H}}^+$  between 1.0 and 13.0.

The straight line represents a mixture of *absolute neutrality*, or, in other words, pure water. The product of the concentrations of the hydrogen and hydroxyl ions in aqueous solutions is, in accordance with accepted views of mass action, constant, and its value, experimentally determined, is  $10^{-14.14}$ . Utilising this fact, the hydroxyl ion concentration may be simply calculated from the hydrogen ion concentration, and this indirect method is the best at present available for the determination of  $p_{\text{OH}}^-$ . Representing by  $p_{\text{OH}}^-$  the negative

exponent of the hydroxyl ion concentration then we may write that for aqueous solutions

$$p_{\text{OH}^-} = 14.14 - p_{\text{H}^+}$$

This relationship is expressed in the diagram. On the right hand side the numerical value of the negative exponent of the hydroxyl ion concentration may be read off directly.

On the left are drawn rectangular spaces each indicating the range of hydrogen ion concentration over which the indicator represented by that space changes its tint. Litmus, for example, it is seen, shows colour changes from about  $p_{\text{H}^+} = 4.5$  to  $8.3$ . The relationship of indicators one to another is shown at a glance, and it can be seen, at once, which indicators will be sensitive to slight changes of ionic concentration for any given value of  $p_{\text{H}^+}$ .

Sørensen has examined many indicators, and has divided them into five groups. The ranges of these groups are represented by the rectangles on the extreme left.

At the right of the diagram I have introduced the results of a few observers on the values of  $p_{\text{H}^+}$  for some of the more familiar physiological fluids.

Certain limitations of the colorimetric method of determining  $p_{\text{H}^+}$  must be recognised and corresponding precautions taken if reliable results are to be obtained. As these are dealt with under the heading 'Sources of error of the method' in Sørensen's paper, their repetition here is unnecessary.

An attempt has been made in the chart, by broken lines at the end of the frame in which the words 'congo-red' are placed, to show the peculiar nature of this indicator.

The chart was first prepared for my own use in connection with a method for titrating pigmented physiological fluids.<sup>1</sup> Its convenience for reference to laboratory workers who have to consider the behaviour of solutions whose reactions are nearly neutral, and the possibility of its use to teachers elucidating the theory and uses of indicators, have led me to its publication.

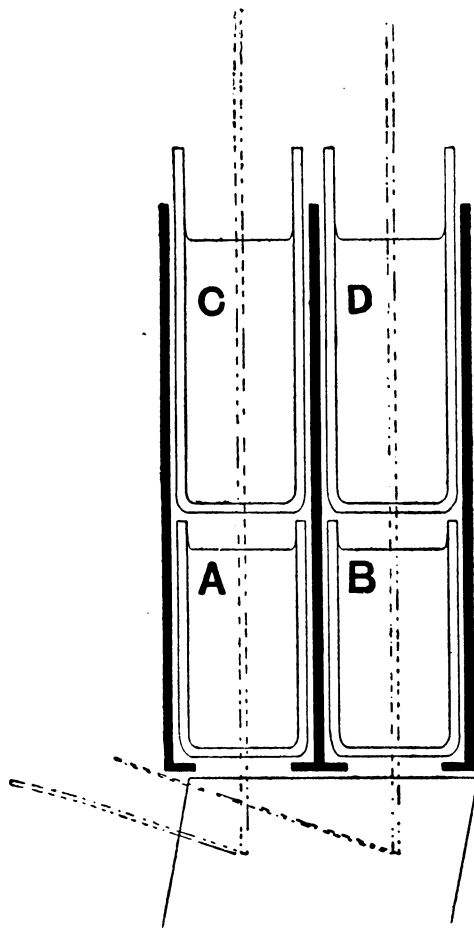
I have added a few examples illustrating the use of the chart.

*EXAMPLE I.—The determination of the hydrogen ion concentration of a given sample of urine.*

Small quantities of urine are placed in each of a series of test tubes, and trials made with a few indicators. It is found to be within the limits of the colour range of litmus and neutral

1. *Proc. Physiol. Soc.*, Vol. XL, p. 27, 1910.

red, but is just outside that of alizarin. Reference to the chart informs us that it is also within the region of colour change of rosolic acid, and, in fact, a number of the indicators of Group III of Sørensen's classification. This is the group he calls the 'Group of the phosphate indicators.' Also, that the mixtures of ' $\text{Na}_2\text{HPO}_4$ ' and ' $\text{KH}_2\text{PO}_4$ ' will serve as standard mixtures in this case. Other mixtures such as 'glycocoll' and ' $\text{NaOH}$ ,' 'sodium citrate and  $\text{HCl}$ ,' will not do; the former because they are too alkaline, the latter because they are too acid. The choice of



indicator will depend upon the approximate value of  $p_{\text{H}}^{\text{c}}$ , and the colours of the indicator and the fluid under investigation, due regard being paid, also, to the 'sources of error' to which reference has already been made. In this instance litmus is chosen as the indicator, and the phosphate mixture is chosen as the standard. As the liquid is pigmented, it will be necessary to adopt either the technique of Sørensen where neutral dyes are used to compensate for the colour of the fluid under investigation or the method used by myself where a second example of the same fluid compensates for its colour.

Using the technique of Sørensen, 10 c.c. of urine are taken in a test tube, and it is found after trial with various neutral dyes that three drops of Bismark Brown solution, and one drop of Primrose Yellow solution, added to 10 c.c. of distilled water match it exactly. A number of mixtures are prepared in test tubes.

	1	2	3	4	5	6
' $\text{KH}_2\text{PO}_4$ '	2.5 c.c.	2.0 c.c.	1.5 c.c.	1.0 c.c.	0.5 c.c.	0 c.c.
' $\text{Na}_2\text{HPO}_4$ '	7.5 c.c.	8.0 c.c.	8.5 c.c.	9.0 c.c.	9.5 c.c.	10 c.c.

Three drops Bismark Brown, one drop Primrose Yellow, and three drops of litmus in all tubes.

To 10 c.c. of the urine in a similar tube three drops of litmus are added, and it is found that it matches Tube No. 1 above.

If, instead of using dyes, the modification of this method which I have adopted be employed the procedure is as follows.

Four glass tubes, A, B, C, D, are arranged as indicated in the diagram in a sort of box with blackened inner walls. Two diaphragms are fitted below, so that a white surface may be seen looking either down C and A or D and B. In A and B are placed 10 c.c. of urine, and in B and C equal quantities of litmus solution. In D 10 c.c. of water are placed, and to C are added the phosphate solutions from burettes in such proportions that when the volume of phosphate mixture reaches 10 c.c. the colours seen on looking down CA and DB match. It is, perhaps, more convenient to prepare some tubes, C', C'', C''', with slightly differing mixtures of phosphates, and to pick out the one that matches most closely. It is found, as before, that 2.5 c.c. ' $\text{KH}_2\text{PO}_4$ ' and 7.5 c.c. ' $\text{Na}_2\text{HPO}_4$ ' solutions fulfil these conditions.

As a result of either operation it is concluded that a mixture of 2.5 c.c. of ' $\text{KH}_2\text{PO}_4$ ' and 7.5 c.c. of ' $\text{Na}_2\text{HPO}_4$ ' behave to litmus as the urine does under conditions designed to determine the hydrogen ion concentration. On referring to the chart it is seen that the value of  $p_{\text{H}}^+$  for this particular phosphate mixture is 7.2, and it is concluded that this is also the value for the sample of urine.

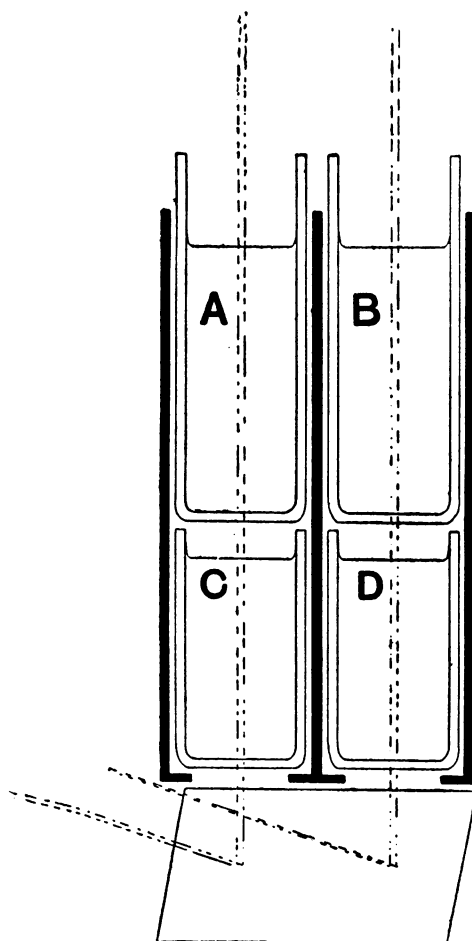
#### EXAMPLE II.—*The titration of a given sample of urine.*

If a titration is to be performed it is necessary to choose an 'end-point' corresponding to a certain value of  $p_{\text{H}}^+$ . The value naturally chosen in many cases is 7.07, that for pure water. It is convenient for use with litmus, as it falls within its range.

For the present purpose the value 9.2, that of ' $\text{Na}_2\text{HPO}_4$ ' solution, might be chosen as end-point were it not that the precipitation of calcium phosphate adds a complication to the process. Let the value 4.5, that of ' $\text{KH}_2\text{PO}_4$ ' be chosen. It is required then to discover how much decinormal hydrochloric acid solution must be added in order to decrease<sup>1</sup> the value of  $p_{\text{H}}^+$  from its original value (we have determined it to be 7.2) to 4.5. It must not be forgotten that the tint of the pigment frequently changes (as in this particular case) with the reaction of the medium, so that in the process of *titration* the duplicate tube must match the solution *after* titration. This factor does not, however, enter into the determination of the hydrogen ion concentration.

1. In accordance with the convention with regard to the omission of the negative sign, the word *decrease* has been used here. It is obvious that the addition of acid to the solution will increase the absolute value of  $p_{\text{H}}^+$ , although the integral value of the exponent will be decreased, since it is a negative quantity.

Using the apparatus mentioned above, the tubes A, B, C, and D are arranged



In C are placed 10 c.c. of ' $\text{KH}_2\text{PO}_4$ ', the value of  $p_{\text{H}}^+$  for which is known to be 4.5, together with three drops of litmus. In D 10 c.c. of water are placed. In A and B are 10 c.c. of urine. Three drops of litmus are also added to B, and then N/10 HCl solution is run in till an approximate correspondence is seen on looking down the two columns. It is found that 2.8 c.c. bring about this result. The like volume, 2.8 c.c., is now run into A, so that the natural pigment in A is made to have the same colour as it has in B. A slight addition to B will now result in a good match, and finally it is found that 2.9 c.c. added to both A and B give the desired result. It is, therefore, stated that, within the limits of error involved in the method, the titration value of 10 c.c. of this sample of urine is 2.9 c.c. N/10 HCl when the end-point chosen is  $p_{\text{H}}^+ = 4.5$ .

Certain statements, some of them of an empirical nature, relating to indicators will perhaps be understood and memorised more readily after reference to the chart. Some examples are given:—

‘Phenolphthalein titrates even weak acids’ is a mode of expressing the fact that the pink colour of this indicator does not appear till the negative exponent of the hydrogen ions in the solution is large. Solutions having the value  $p_{\text{H}}^+$  between, roughly, 7.5 and 8.0 are said to be ‘acid to phenolphthalein and alkaline to litmus.’ Referring to the testing of new glass for liberation of alkali in contact with water it is frequently stated that ‘Rosolic acid is extremely sensitive to alkalis.’ This is readily understood when it is seen that its colour change occurs when the logarithm of the hydrogen ion concentration is just numerically greater than that of distilled water. The colloidal nature of Congo-red renders its behaviour exceptional, and for the purpose of the determination of  $p_{\text{H}}^+$  colorimetrically it is useless in that the results are inaccurate. Roughly speaking, however, its position in the diagram indicates that it ‘reacts to strong acids, and not weak ones,’ and its relations to  $p_{\text{H}}^+$  values are similar to those of the indicators of Group I.

In collecting results of various observations of  $p_{\text{H}}^+$ , it is not to be forgotten that these are logarithms and the arithmetical mean value of the logarithms is the geometrical mean of the corresponding concentrations.